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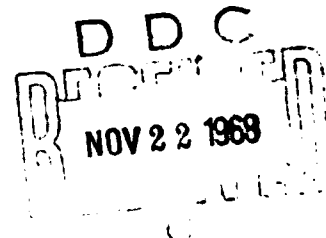
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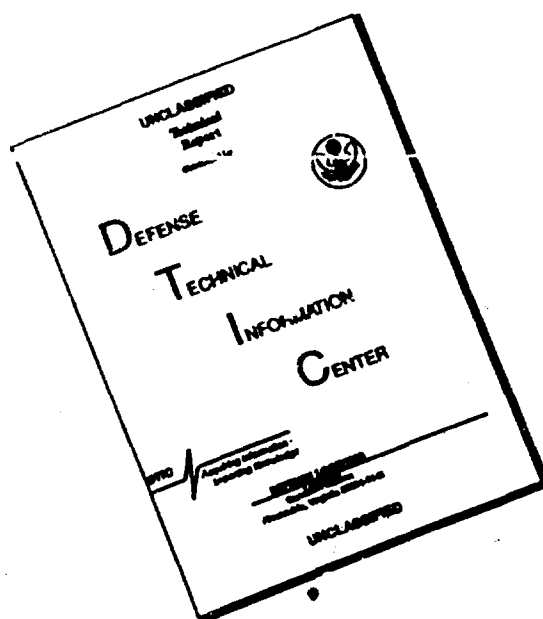
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HYDRATION OF MACROMOLECULES
IN THE NATIVE AND DENATURED STATES

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It is well known that the presence of water has a strong effect on the structure and property of macromolecules. A wet macromolecule is far from being the same thing as a dry one. Judging from the curves of sorption and desorption in proteins and nucleic acids they do not behave as rigid, inert substrata, but rather as deformable reactive substances changing their structure under the effect of water [1, 2].

The effect of water on the macromolecule, however, is not unilateral and, in turn, it is possible only as the result of change in the structure of the water itself under the action of the macromolecule.

The different groups in the macromolecule structure -- polar, apolar, and charged -- are known to be able to act in different ways on water structure by raising or lowering its degree of order [3, 4]. The conformation of the macromolecules, however, is on the whole determined by the efficiency of the various contacts, i.e., the condition of minimum total free energy in the system.

There are at present great discrepancies in evaluating the thermodynamic effects of the reaction of the different groups with water. For the most part this involves the apolar groups, which exert a strong ordering effect on water [5] and at the same time occur in large amounts in macromolecules. The varying estimates of the thermodynamic characteristics of their interaction with water has led to two views, opposed to each other in the extreme, of the effect of macromolecules on water and of the factors determining their conformation.

According to Kauzmann [6] the ordering effect of apolar groups

on water is not thermodynamically efficient and therefore the macromolecule tends to adopt a compact conformation in which the number of contacts between apolar groups and water is minimum, while at the same time its ordering effect on the water is also minimum.

The opposite view developed by Klotz [7-9] proceeds from the assumption that the ordering effect of the apolar groups on water is thermodynamically effective and that the macromolecule conformation will be most stable which permits the greatest ordering of the water. Starting from this model the denaturation of macromolecules represents the destruction of extensive ordered water layers alongside the macromolecule -- the "melting of the ice-like framework" supporting its structure. In other words, in this case denaturation must be accompanied by reduced hydration of the macromolecule, while, as according to Kauzmann, denaturation should lead to heightened hydration since in this process the number of contacts between apolar groups and water grows larger.

Although Kauzmann's view has recently become more widespread [10] it still cannot be considered unquestioned, and in solving this problem the start must obviously be made from specific findings on macromolecule hydration.

Hydration of Native Macromolecules

There is at present a great deal of experimental material on hydration of macromolecules since for these purposes practically all the methods sensitive to changes in state of water have been employed.

The amount of bound water was calorimetrically measured from the wetting energies [11], from the compressibility by ultrasonic sounding [12, 13], from x-ray dispersion [14-16], from the autodiffusion rate of the water [17, 18], from the electrical conductivity [19], and, finally, by means of nuclear magnetic resonance (NMR) [20-26] and high-frequency permittivity [27-32].

In the calculation of hydration conducted by different authors in diverse ways, however, there are substantial discrepancies indicating either low sensitivity of the method or that the measured quantity was after all not so directly connected with the state of the water as might seem when proceeding from a simplified model.

This observation refers above all to the NMR method which has recently become widespread and whose findings have led different authors to completely opposite conclusions.

Jacobson [20], for example, believes that the NMR method enabled him to confirm the existence of coordinated water in solutions of DNA. Later in considerably improved instruments, however, it was impossible

to discover any convincing indications of the existence of bound water in DNA solutions [21, 26]. In similar fashion the existence of bound water was shown by the NMR method in agar gels by Hechter [22], but Balass et al. [21] came to a negative result.

The same may be said also of the data on hydration of globular proteins, e.g., Bovey [23] is of the opinion that serum albumin leads to no appreciable rise in the number of H bonds in water, but the authors of Reference 25 arrive at a completely opposite conclusion and consider that in solutions of egg albumin (which in this respect cannot be qualitatively differentiated from serum albumin) there are rigidly bound water molecules, while in denaturation their number even increases.

Usually most of the data on NMR which might be regarded as testifying in favor of the existence of ordered water in macromolecule solutions may after critical analysis prove to be merely artifacts. Thus, the cause of the widening of the line in macromolecule solutions may also be increased viscosity [26] and diamagnetic anisotropy [21], and even ferromagnetic anisotropy in the case of DNA solutions [33]. The sensitivity of this method even in the most recent works is calculated at 5% [26]; therefore it is in general not surprising that use of this method was not even once able to give reliable data on water.

Hydration estimated from the degree of high-frequency permittivity apparently gives better results, but the error in determining the quantity of bound water in solution reaches 2% even in this case [32].

Calorimetry proved to be particularly convenient for determining macromolecule hydration. This method makes it possible to ascertain the quantity of bound water in solution with the greatest possible accuracy at present -- 0.15%. Moreover this method is the most direct, and hence also more unambiguous.

The basic idea of the method, its advantages, and its clarity become clear from the following two figures (Figs. 1 and 2) which represent the temperature dependence of the specific heat of 1 gram of DNA and procollagen in the presence of different amounts of water.

Calorimetric measurements were made on an absolute vacuum adiabatic calorimeter with a bulb of 0.8 cc volume. Into the bulb was put 50 to 100 mg of the preparation and the necessary amount of water. The specific heat measured was converted either for grams of dry weight or the partial specific heat of the water in solution was computed. Accuracy in determining the specific heat and melting points ensures that the quantity of bound water will be found to an accuracy of up to 1 mg in 800 mg of the total amount of water. A previous article [34] describes the calorimeter design, the method of processing the data, and the computation of corrections in great detail.

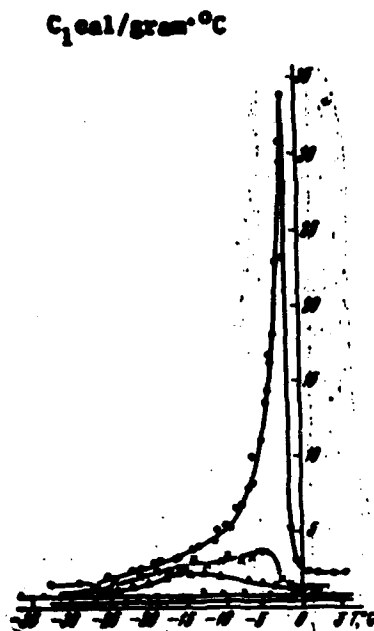


Fig. 1. Temperature Dependence of Specific Heat of 1 gram of DNA in Presence of Different Amounts of Water (0.05, 0.75, 1.00, and 2.00 gram)

considerably diffuse with respect to temperature and shifted from 0°C towards low temperatures. This indicates that the water which freezes and consequently is free, undergoes a severe effect of macromolecule charges.

With increase in the amount of water the heat absorption hump develops into a peak and is displaced toward 0°C, which indicates the occurrence of layers of water which are under less influence from macromolecules.

It is noteworthy that the shape of the water melting curve is directly dependent on the nature of the macromolecule. As Fig. 3 shows, thermal absorption in the case of globular proteins is spread over a considerably narrower temperature range than in the case of fibrillary procollagen. In solutions of DNA, however, melting begins at even lower temperatures. This effect is apparently conditioned by the extent of contacts between macromolecules and water, the charge concentration on the macromolecule surfaces, and the size of the individual charges (in

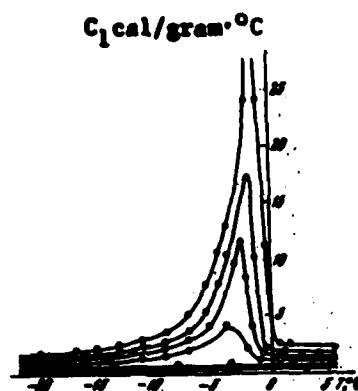


Fig. 2. Temperature Dependence of Specific Heat of 1 gram of Procollagen in Presence of Different Amounts of Water (0, 0.35, 0.64, 0.93, 1.26, and 2.00 gram)

0.5 gram per gram of dry weight of DNA and and 0.3 gram per dry weight of procollagen does a hump of heat absorption occur, indicating that there are already H bonds in the water which are capable of being frozen or unfrozen by a change in temperature. The observed heat absorption is, however, con-

As is evident from the figures the addition of water up to a certain limit leads to a certain absorption of heat in the 0°C region. Consequently all the water added passes over into a state which does not freeze on cooling nor become fluid on subsequent heating; in other words, it is in a bound state or, more exactly, in an ordered state.

Only when water is added in an amount of more than

particular in the case of DNA, melting in the -25°C is, as evident, connected with the existence of phosphate charges.

With respect to the heat of melting or to observable area of the peak the amount of water in the solution which melts when the solution thaws, and consequently also the amount of bound water may be calculated and the effect of the macromolecules on the water estimated.

Table I gives the results of this sort of computation for different macromolecules

Table I. Macromolecule Hydration

(a) Препарат	(b) Гидратация калориметрически с H_2O в сухого веса ± 0.005	(c) Стабилизация слоем H_2O слоем зонта	(d) Толщина гидратного слоя, Å	(e) Гидратация (по данным литературы) с H_2O в сухого веса	(f) Метод
(g) ДНК	0,610	11,80	4,0	0,35	самодиффузия [17] (j')
(h) Прокollaген	0,465	2,40	2,8	0,40	теплота смачивания желей- типа [11] (k)
(i) Сывороточ- ный альбу- мин	0,320	2,05	3,2	0,35 0,30 0,38	изотермы сорбции [35] (l) ультразвук [13] (m) диэлектрическая постоян- ная [31] (n)
(j) Яичный аль- бумин	0,323	2,06	3,4	0,30 0,37 0,58 0,34 0,246	ультразвук [13] (m) рентгенограф [13] (o) рентгенограф [14] (o) диэлектрическая постоян- ная [29] (n)
(k) Гемоглобин	0,324	2,07	3,8	0,36 0,18 0,55 0,35 0,22 0,30	ультразвук [13] (m) (j') самодиффузия [18] (j') ультразвук [13] (m) электропроводность [19] (p) рентгенограф [16] (o) диэлектрическая постоян- ная [32] (n)

Key: (a) Preparation, (b) Hydration calorimetrically determined, ratio of grams of H_2O to grams of dry weight ± 0.005 , (c) Stabilization, ratio of moles of H_2O to moles of the group, (d) Thickness of hydrated layer, Å, (e) Hydration (according to data in literature), ratio of grams of H_2O to grams of dry weight, (f) Method, (g) DNA, (h) Procollagen, (i) Serum albumin, (j) Egg albumen, (k) Hemoglobin, (j') Autodiffusion, (k) Wetting heat of gelatin, (l) Sorption isotherms, (m) Ultrasound, (n) Dielectric constant, (o) Roentgenogram, (p) Electrical conductivity.

The second column gives the macromolecule hydration values in grams of water per gram of dry weight; the third column, the number of moles of bound water per mole of the monomer group; the fourth column, the thickness of the hydrate layer computed from the hydration found and the geometric parameters of the macromolecules under the assumption that

the hydrate layer is compact and evenly covers the whole surface of the macromolecule (which is merely an assumption giving a rough figure for a simplified model of the structured water beside the macromolecules); and the two final columns give the hydration values derived by various methods with the corresponding sources indicated.

We see that macromolecules actually to a considerable degree order the water beside themselves, but this, of course, is the total effect engendered by the action of different mechanisms -- ions binding the water molecules [36], polar groups forming H bonds with them [28, 29, 35], and, finally, apolar groups. From the adduced data it must not be inferred that a certain component has made a contribution, nor even less must anything be said about the models of Kauzmann or Klotz -- i.e., which of them reflects the real situation.* An attempt may also be made to derive some information about this by studying the change in hydration during denaturation of the macromolecules.

Hydration of Denatured Macromolecules

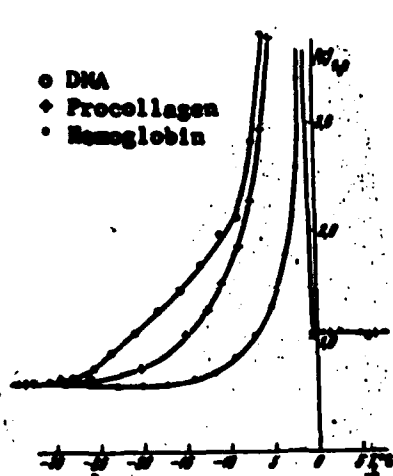


Fig. 3. Temperature Dependence of Partial Specific Heat of Water in Macromolecule Solutions with Concentration of 2 gram of Water per Gram of Dry Weight.

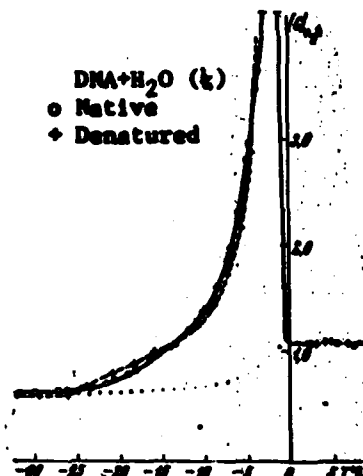


Fig. 4. Temperature Dependence of Partial Specific Heat of Water in Solution of Native and Denatured DNA. Solution contains 4 gram of Water per Gram of Dry Weight.

Available experimental data on the denaturation change in macromolecule hydration are even more conflicting than the estimates of hydration itself.

Some authors find that in denaturation the hydration is reduced [29, 37-40]. Some believe that hydration is stepped up during denaturation [41, 42]; others

incline to the opinion that the denaturation changes in hydration are extremely insignificant [26, 29, 43], but that the effects observed are only artifacts. Thus, measurement of the absorption line observed by

*Note at foot of page 7.

NMR in DNA solutions when the temperature rises is caused not by the change in water structure, but merely by the occurrence of air bubbles [26].

Taking into consideration the high sensitivity of the calorimetric method of determining hydration it was possible to expect that it would give more reliable information on the given problem.

Figures 4, 5, and 6 give the curves of water melting in solutions of native and denatured macromolecules. As is apparent, very essential changes in distribution of charges and water contact with the macromolecule surface occur. The impression is produced that some of the ions pass from the macromolecules into the solution and reduce its melting temperature. In the case of DNA solutions other authors, proceeding from a study of the change in electrical properties of the solutions, have come to the same conclusion that denaturation is accompanied by a restructuring of the ionic atmosphere (e.g., see [44, 45]).

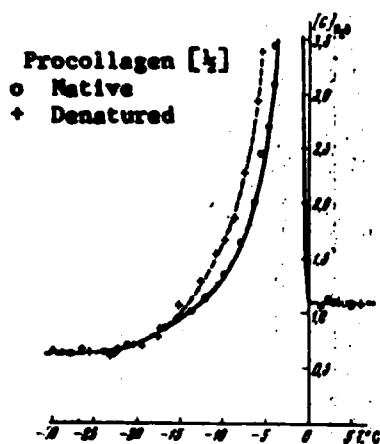


Fig. 5. Temperature Dependence of Partial Specific Heat of Water in Solution of Native and Denatured Procollagen (2 gram of water per gram of dry weight)

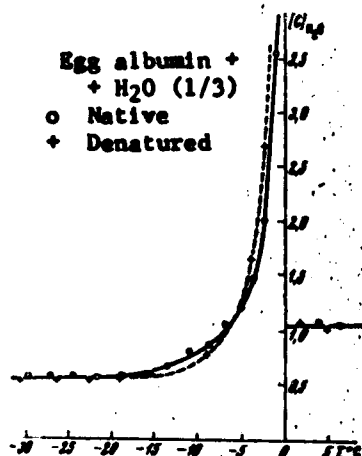


Fig. 6. Temperature Dependence of Partial Specific Heat of Water in Solution of Native and Denatured Egg Albumin (3 gram of water per gram of dry weight)

In addition to the change in shape of the melting curves, however, their area also changes, and hence also hydration.

The results of calorimetric determination of hydration in native and denatured preparations are summarized in Table II.

As the table shows, a certain rise in hydration

*It must be noted that the hydration indexes of the globular proteins which we determined are very close to the values obtained by Fisher [46] proceeding from the assumption that only polar groups of amino acids are found on the macromolecule surface. This coincidence of experimental with theoretical findings undoubtedly serves as a weighty argument in favor of the assumption that hydration in the native protein proceeds basically at the expense of reaction of water with polar groups.

is observed in all cases, but this change is actually very small.

Table. II. Hydration of Macromolecules in Native and Denatured State

(a)	Препарат	(b) Гидратация нативных препаратов	Гидратация денатуриро- ванных препаратов (c)
(d)	ДНК + вода (1/4)	0,611	0,645
(e)	ДНК + 0,15 M NaCl (1/5)	0,526	0,584
(f)	Прокollaген + 0,15 M цитратный буфер (1/3)	0,485	0,492
(g)	Прокollaген + вода (1/2)	0,465	0,519
(h)	СА + вода (1/3)	0,315	0,330
(i)	ЭА + вода (1/3)	0,323	0,332
(j)	ЭА + 0,15 M боратный буфер, pH 10,0 (1/3)	0,322	0,333
(k)	Гемоглобин + вода (1/3)	0,324	0,339
(l)	Гемоглобин + 0,15 M боратный буфер pH 10 (1/3)	0,336	0,345

Key: (a) Preparation, (b) Hydration of native preparations, (c) Hydration of denatured preparations, (d) DNA + water (k), (e) DNA + 0.15 M NaCl (1/5), (f) Procollagen + 0.15 M citrate buffer (1/3), (g) Procollagen + water (1/2), (h) Serum albumin + water (1/3), (i) Egg albumin + water (1/3), (j) Egg albumin + 0.15 borate buffer, pH 10.0 (1/3), (k) Hemoglobin + water (1/3), (l) Hemoglobin + 0.15 M borate buffer pH 10 (1/3)

entirely impregnated with water. It is very improbable that water completely penetrates the hydrophobic environment and comes into contact with the apolar groups.

There is also a third reason which must undoubtedly have greatly lowered the denaturation effect calorimetrically recorded -- since hydration is determined at a temperature below the denaturation temperature (0°C) a partial "collapse" of the structure must occur, but this collapse does not always mean "denaturation" in the sense of complete restoration of conformation. The aggregation of macromolecules also possibly makes a contribution to reducing the effect.

In one way or another the above findings undoubtedly indicate that the hydration of macromolecules actually changes in denaturation, while this change is always positive -- the hydration of denatured macromolecules is always greater than that of native ones. This experimental fact first unquestionably serves as a corroboration of Kauzmann's model, and second draws our attention to the close interconnection between

Certain considerations may be expressed to explain such a small degree of effect.

First, ordering by apolar groups is not the unique and possible not even the main factor acting upon the water. Therefore, although the ordering effect in denaturation substantially rises, the relative change in total hydration is not great.

Second, in denaturation there is far from a hundred-percent change in the number of bonds in the macromolecule. In particular, in the case of the globular proteins it has hitherto been doubtful whether denaturation may be regarded as the transition of a dense compact formation into a loose chaotic coil

macromolecule conformation and the state of the water in the layers contiguous to it. This situation is usually either neglected or simply lost from sight when examining the conformation transformations of macromolecules in water -- which is hardly to be tolerated.

Conclusions

1. The question of the reciprocal influence of macromolecules and the water layers adjacent to them is examined. The Kauzmann and Klotz models are compared.
2. Experimental findings on macromolecule hydration calorimetrically obtained are cited.
3. The denaturation change of hydration is studied. It is demonstrated that hydration of denatured macromolecules is greater than hydration of native molecules, which speaks in favor of Kauzmann's model.

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